ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2009, p. 2082–2088 Vol. 53, No. 5
0066-4804/09/$08.00+0 doi:10.1128/AAC.01214-08

Quasiexperimental Study of the Effects of Antibiotic Use, Gastric Acid-Suppressive Agents, and Infection Control Practices on the Incidence of *Clostridium difficile*-Associated Diarrhea in Hospitalized Patients▽

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Received 12 September 2008/Returned for modification 13 January 2009/Accepted 1 March 2009

The objective of this study was to evaluate the effects of antimicrobial drug use, gastric acid-suppressive agent use, and infection control practices on the incidence of *Clostridium difficile*-associated diarrhea (CDAD) in a 426-bed general teaching hospital in Northern Ireland. The study was retrospective and ecological in design. A multivariate autoregressive integrated moving average (time-series analysis) model was built to relate CDAD incidence with antibiotic use, gastric acid-suppressive agent use, and infection control practices within the hospital over a 5-year period (February 2002 to March 2007). The findings of this study showed that temporal variation in CDAD incidence followed temporal variations in expanded-spectrum cephalosporin use (average delay = 2 months; variation of CDAD incidence = 0.01/100 bed-days), broad-spectrum cephalosporin use (average delay = 2 months; variation of CDAD incidence = 0.02/100 bed-days), fluoroquinolone use (average delay = 3 months; variation of CDAD incidence = 0.004/100 bed-days), amoxicillin-clavulanic acid use (average delay = 1 month; variation of CDAD incidence = 0.002/100 bed-days), and macrolide use (average delay = 5 months; variation of CDAD incidence = 0.002/100 bed-days). Temporal relationships were also observed between CDAD incidence and use of histamine-2 receptor antagonists (H2RAs; average delay = 1 month; variation of CDAD incidence = 0.001/100 bed-days). The model explained 78% of the variance in the monthly incidence of CDAD. The findings of this study highlight a temporal relationship between certain classes of antibiotics, H2RAs, and CDAD incidence. The results of this research can help hospitals to set priorities for restricting the use of specific antibiotic classes, based on the size-effect of each class and the delay necessary to observe an effect.

Clostridium difficile, a spore-forming gram-positive anaerobic bacillus, is a common pathogen in hospitals, with gastrointestinal colonization giving rise to increased morbidity, mortality, and health care costs (32). The clinical spectrum of *Clostridium difficile*-associated diarrhea (CDAD) ranges from uncomplicated diarrhea to severe life-threatening pseudomembranous colitis (23). Established risk factors for CDAD include host factors (for example, advanced age and comorbidities [21, 28]), poor infection control practices (relating to the health care environment, health care workers' hand hygiene, etc.) (4, 21), exposure to factors that disrupt the normal protective intestinal microflora (i.e., broad-spectrum antibiotics) (6, 24, 25), and the use of gastric acid-suppressive agents, i.e., proton pump inhibitors (PPIs) (1, 8, 9) and histamine-2 receptor antagonists (H2RAs) (10). In a systematic review, which was undertaken to summarize the strength of the evidence for a relationship between antibiotic use and the occurrence of CDAD, most studies cited were limited in their ability to establish a causal relationship due to the presence of bias, small sample sizes, and the inadequate control of confounding factors (31). The authors of the review concluded that well-designed studies are needed to identify true risk factors for CDAD and to provide reliable estimates of the strength of association (31).

The objective of the present study was to combine data on the use of antibiotic agents, PPIs, and H2RAs and infection control practices in order to comprehensively evaluate temporal relationships between these factors and CDAD incidence over time. Since temporally sequenced observations of CDAD and antimicrobial drug use are not independent, applying simple regression analysis would be inappropriate to evaluate such data (2, 29). A time-series analysis was therefore used to transform our data into a series of independent values and to examine the trends and autocorrelations over time, including characteristics for each explanatory variable and the outcome of interest (CDAD incidence).

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▽ Published ahead of print on 16 March 2009.

MATERIALS AND METHODS

Setting and study period. The study was carried out in the Antrim Area Hospital in Northern Ireland, United Kingdom, a 426-bed district general teaching hospital serving a population of approximately 420,000. The hospital provides all acute, general medical, and surgical services; supports a range of outpatient facilities; and acts as a center for the coordination of health service provision throughout a defined geographical area in Northern Ireland. The present retrospective investigation involved collecting data on a monthly basis on the usage of antibiotics and gastric acid-suppressive agents and on infection control practices together with the incidence of CDAD within the hospital over a 5-year period (February 2002 to March 2007). The study was ecological in design.

Microbiology and pharmacy data. The number of CDAD cases (on a monthly basis) was obtained from the clinical microbiology information system over the study period. Duplication was removed from these data such that more than one positive *C. difficile* test from the same patient was considered as a single episode if the positive tests were ≤28 days apart. Within the hospital laboratory, clinical samples were processed according to routine microbiology procedures. The presence of *C. difficile* was identified via the detection of toxins A and B directly from the feces of patients with colitis-like symptoms. The microbiology laboratory utilizes the Premier Toxin A and B kit, an enzyme-linked immunosorbent assay technique; the standard methodology supplied with the kit was used throughout the study period. The low sensitivity of the enzyme-linked immunosorbent assay was recognized; therefore, it was standard operational hospital policy for patients who had diarrhea and an initial toxin-negative test to have subsequent stool samples, collected at least 8 hours apart, retested to a maximum of three samples.

Prior to January 2005, it was hospital policy to test samples of feces for *C. difficile* only when this was specifically requested by the physician. From the beginning of January 2005, in accordance with new government guidelines, the testing of feces from all patients of 65 years of age and over with diarrhea was introduced, while physician-requested testing continued in patients of ≥2 years of age.

Bed occupancy data over the study period were obtained at monthly intervals to calculate the incidence of CDAD per 100 bed-days. For the purpose of this study, CDAD was defined as a toxin-positive test plus diarrhea (an increased number [two or more] of watery/liquid stools [i.e., types 6 and 7 according to the Bristol Stool Scale (16)] that is greater than normal for the patient, within the duration of 24 h). The definition excluded children less than 2 years old. The monthly quantities of each antibiotic delivered for patient care to each ward of the hospital were obtained from the hospital pharmacy information system. These quantities were converted into defined daily doses (DDDs) following the recommendations of the World Health Organization (WHO) (35). The numbers of DDDs of individual antibiotics were then grouped into classes belonging to group J01 (antibacterials for systemic use) of the Anatomical Therapeutic Chemical classification system from the WHO Collaborating Center for Drug Statistics Methodology and were finally expressed as a number of DDDs per 100 bed-days (35). The same approach was taken for PPIs and H2RAs.

Infection control practices. Data were also collected on the monthly quantities of chlorhexidine (liters) and alcohol-based hand rub (ABHR) (liters) dispensed, again using the hospital pharmacy information system. The latter parameters were finally expressed as volumes per 100 bed-days. Additionally, data relating to staffing levels of nurses/auxiliary nurses were collected on a monthly basis over the 5-year study period and expressed as whole-time equivalents per 100 bed-days.

Statistical analysis. Autoregressive integrated moving average (ARIMA) models, using the Box-Jenkins method for analysis (14), were used to evaluate whether relationships existed between antibiotic use, the use of gastric acid-suppressive agents, and the level of infection control practices and the incidence of CDAD. By using multivariate transfer function models, the association between the "explanatory" time series of usage of antibiotics, H2RAs, PPIs, and ABHR and the "response" time series of the monthly incidence density of CDAD was assessed, taking into account the possible time delays (of up to 5 months) of the effects of the explanatory variables.

A transfer function model, which consists of modeling a time series as a function of its past values and random errors, was built. For each individual series, an ARIMA model was identified and fitted according to the Box-Jenkins methodology (14). At the outset, the series were checked for stationarity (i.e., having a constant mean and variance) using the augmented Dickey-Fuller test for unit roots. Following this, the model was identified by determining the ARIMA model orders (p, d, q) using autocorrelation and partial autocorrelation. The model parameters were then estimated by the unconditional least-squares method. Finally, the adequacy of the model was checked and the statistical significance of the parameters was determined. Among different models, the most parsimonious one was chosen with the fewest parameters, the lowest Akaike information criterion, and the best R^2. The generated coefficient (R^2) measures the overall fit of the regression line, i.e., the fraction of the variance of the dependent variable explained by the regression model.

After identification of the multivariate transfer function models, the cross-correlation function was determined by estimating the correlations between the series describing antibiotic, H2RA, PPI, and ABHR use at different time lags and the CDAD series. Significance tests for parameter estimates were used to eliminate the unnecessary terms in the model. A P value of 0.05 was considered to be statistically significant. All statistical analyses were performed using EViews 3 software (QMS, Irvine, CA).

RESULTS

Over the 5-year study period, there were 393 CDAD cases identified out of a total of 203,296 admissions. The average observed monthly CDAD incidence was 0.06/100 bed-days (range, 0.00 to 0.17). Trends in the use of each class of antibiotic and gastric acid-suppressive agent are presented in Table 1. The use of some antibiotic classes remained constant during the study period, whereas other classes, e.g., combinations of penicillins with beta-lactamase inhibitors (mostly amoxicillin-clavulanic acid), macrolides, and fluoroquinolones, showed a significant increasing trend in their use. Similarly, analysis of the data showed a significant positive trend for some infection control practices, i.e., number of samples tested for *C. difficile* and nursing/auxiliary nursing levels, whereas other practices remained fairly stable (Table 2).

Multivariate time-series analysis showed significant relationships between the incidence of CDAD and a number of potential explanatory variables. Statistically significant positive relationships were observed for the use of expanded-spectrum cephalosporins, broad-spectrum cephalosporins, fluoroquinolones, amoxicillin-clavulanic acid, macrolides, and H2RAs with various time lags (Table 3). The model showed that temporal variations in CDAD incidence followed temporal variations in expanded-spectrum cephalosporin use with an average delay of 2 months. This means that, on average, an increase (or decrease) of expanded-spectrum cephalosporin use by 1 DDD/100 bed-days resulted 2 months later in an increase (or decrease) of the incidence of CDAD by 0.01/100 bed-days. Effects of different sizes with different delays were observed for broad-spectrum cephalosporin use (average delay = 2 months; variation of CDAD incidence = 0.02/100 bed-days), fluoroquinolone use (average delay = 3 months; variation of CDAD incidence = 0.004/100 bed-days), amoxicillin-clavulanic acid use (average delay = 1 month; variation of CDAD incidence = 0.002/100 bed-days), and macrolide use (average delay = 5 months; variation of CDAD incidence = 0.002/100 bed-days) (Table 3). Temporal relationships were also observed between CDAD incidence and use of H2RAs (average delay = 1 month; variation of CDAD incidence = 0.001/100 bed-days) (Table 3). No correlation was found between PPI use, nursing levels, and infection control practices and the incidence of CDAD.

Three stochastic terms were introduced into the model, i.e., an autoregressive term with a lag time of 4 months, a moving average term with a lag time of 1 month, and a seasonal moving average term with lag time of 12 months (Table 3). Those terms reflected autocorrelation in the incidence of CDAD, i.e., this incidence was related to the incidence observed in the

TABLE 1. Characteristics of the monthly antimicrobial, PPI, and H2RA use in the Antrim Area Hospital (February 2002 to March 2007)

Antimicrobial class (ATC[a] group)	Avg monthly use in DDDs/100 bed-days (range)	Trend	
		Coefficient	*P* value
Tetracyclines (J01A)	0.85 (0.00–2.68)	0.01	0.013
Penicillins with extended spectrum (J01CA)	2.64 (1.29–5.32)	−0.002	0.690
Beta-lactamase-sensitive penicillins (J01CE)	0.86 (0.13–2.03)	−0.007	0.031
Beta-lactamase-resistant penicillins (J01CF)	3.17 (1.33–6.28)	0.004	0.551
Combinations of penicillins including beta-lactamase inhibitors (J01CR)	24.50 (15.25–35.85)	0.267	0.043
Narrow-spectrum cephalosporins (J01DB)	0.34 (0.01–0.92)	−0.002	0.402
Expanded-spectrum cephalosporins (J01DC)	2.82 (1.24–4.66)	0.014	0.539
Broad-spectrum cephalosporins (J01DD)	0.61 (0.20–1.21)	−0.001	0.517
Carbapenems (J01DH)	0.42 (0.00–3.00)	0.016	<0.0001
Trimethoprim and derivatives (J01EA)	1.69 (0.73–2.67)	−0.008	0.103
Combinations of sulfonamides and trimethoprim including derivatives (J01EE)	0.21 (0–1.00)	0.003	0.135
Macrolides (J01FA)	9.96 (5.64–21.37)	0.103	<0.0001
Lincosamides (J01FF)	0.41 (0.00–1.20)	0.005	0.008
Aminoglycosides (J01GB)	1.03 (0.35–2.56)	−0.0013	0.0004
Fluoroquinolones (J01MA)[b]	6.95 (3.35–12.05)	0.095	<0.0001
Glycopeptide (J01XA)	1.18 (0.29–2.46)	0.019	<0.0001
Steroid antibacterials (J01XC)	0.84 (0.19–2.05)	0.011	<0.0001
Imidazole derivatives (J01XD)	4.25 (2.56–7.37)	0.03	0.001
Nitrofuran derivatives (J01XE)	0.26 (0.00–0.88)	0.004	0.001
Other antibacterials (J01XX)	0.24 (0.00–1.00)	0.006	<0.0001
Antibacterials for systemic use, total (J01)	63.32 (41.15–88.46)	0.466	<0.0001
PPIs (A02BC)	56.73 (23.28–99.02)	1.279	<0.0001
H2RAs (A02BA)	7.99 (3.01–40.69)	0.019	0.564

[a] ATC, anatomical therapeutic chemical.
[b] Mostly ciprofloxacin (J01MA02).

previous months. The determination coefficient (R^2) of the final model was 0.78, i.e., 78% of the variation in the monthly incidence of CDAD over the study period was explained by the factors included in the model. Projections for Antrim Area Hospital on the DDDs of the implicated agents and the numbers of patients needed to be treated to cause or prevent one CDAD case at the hospital are presented in Table 4.

Graphical representations of the relationships between the monthly use of expanded-spectrum cephalosporins, broad-spectrum cephalosporins, fluoroquinolones, amoxicillin-clavulanic acid, macrolides, and H2RAs and the monthly incidence of CDAD are presented in Fig. 1. In this, data were plotted using a 5-month moving average transformation, i.e., the value plotted for a specific month is the average of the value observed this month, the two previous months, and the two following months.

Finally, a curve of the summed monthly use of all explanatory variables, taking into account their respective lags, was constructed and plotted on the same graph as the monthly incidence of CDAD (Fig. 2). This showed the parallel nature of the relationship between these lagged explanatory variables and the incidence of CDAD at the Antrim Area Hospital and provided visual confirmation of the model.

DISCUSSION

The main objective of this research was to model the impact of antibiotics, gastric acid-suppressive agents, and infection control practices on the incidence of CDAD in hospitalized patients. The study showed temporal relationships between the use of certain antibiotic classes and H2RAs, and the incidence of CDAD. The use of expanded-spectrum cephalosporins, broad-spectrum cephalosporins, fluoroquinolones, amoxicillin-clavulanic acid, and macrolides was positively correlated with

TABLE 2. Characteristics of the monthly infection control practices and related factors in the Antrim Area Hospital (February 2002 to March 2007)

Variable (measurement unit)	Monthly avg (range)	Trend	
		Coefficient	*P* value
Infection control practices			
ABHR (liters/100 bed-days)	0.27 (0.00–4.01)	−0.004	0.433
Chlorhexidine (liters/100 bed-days)	1.22 (0.90–1.80)	0.005	0.094
No. of samples tested for *C. difficile* (no./100 bed-days)	2.98 (1.66–4.46)	0.0029	<0.0001
Other variables			
Nursing levels (whole-time equivalents/100 bed-days)	3.12 (2.14–3.72)	0.009	<0.0001
Auxiliary nursing levels (whole-time equivalents/100 bed-days)	0.95 (0.54–1.18)	0.003	0.004

TABLE 3. Multivariate time-series analysis model for monthly CDAD incidence ($R^2 = 0.78$)

Term	Lag time[a] (mo)	Coefficient (SE)[b]	*T* ratio	*P* value
Use of drugs (DDDs/100 bed-days)				
Expanded-spectrum cephalosporins	2	0.010299 (0.003365)	3.061	0.0038
Broad-spectrum cephalosporins	2	0.018226 (0.006284)	2.900	0.0059
Fluoroquinolones	3	0.003835 (0.001139)	3.367	0.0016
Amoxicillin-clavulanic acid	1	0.001518 (0.000471)	3.223	0.0024
Macrolides	5	0.001835 (0.000826)	2.221	0.0317
H2RAs	1	0.001035 (0.000335)	3.093	0.0035
AR[c]	4	0.775923 (0.059765)	12.983	<0.0001
MA[d]	1	0.558183 (0.142460)	3.918	0.0003
SMA[e]	12	−0.933954 (0.016596)	56.277	<0.0001

[a] Represents the delay necessary to observe the effect.
[b] Indicates the size and the direction of the effect.
[c] AR, autoregressive term, representing past incidence density of CDAD.
[d] MA, moving average term, representing past disturbances in the incidence density of CDAD.
[e] SMA, seasonal moving average term, representing seasonality in past disturbances in the incidence density of CDAD.

the incidence of CDAD. The findings were consistent with those reported by others in relation to the role of the antibiotics in increasing CDAD incidence rates in hospitals (3, 6, 7, 12, 15, 19, 24–26, 30, 31).

Given the extensive background knowledge regarding the possible lines of evidence between antimicrobial use in hospitals and resistance which were proposed by McGowan (22), we were able to demonstrate evidence that might be consistent with cause-effect relationships which followed both increases and decreases in antibiotic use. Although resistance of *C. difficile* to certain antibiotics may play an important role in CDAD development, it is presumed that an antimicrobial promotes *C. difficile* infection by disrupting the indigenous microflora of the colon, which provides an important host defense against colonization by, and overgrowth of, *C. difficile* and other pathogens (25). Although a low volume of use of both clindamycin and expanded-spectrum/broad-spectrum cephalosporins (both of which are considered risk factors for CDAD) was observed, a significant association was established only with the latter. Such findings required further investigations.

As previously reported by others (10), the results of this analysis showed a significant correlation between the use of H2RAs and the subsequent incidence of CDAD. A possible explanation for such findings is that these agents may suppress gastric acid, thus increasing host susceptibility to upper gastrointestinal tract infections (10). Although a number of studies have demonstrated an association between CDAD and the use of PPIs (1, 8, 9), our findings showed that there was no such significant association, confirming similar findings in several other studies (18, 20, 26). The reason why one group of the gastric acid-suppressive agents (i.e., H2RAs) was positively correlated with the CDAD incidence while the other major class of agents (i.e., PPIs) was not remains unclear and requires further investigation with a larger sample size.

In addition to antibiotic and H2RA use, the model included surrogate markers of infection control practices, i.e., the use of ABHRs and chlorhexidine and the number of samples tested for *C. difficile*, together with the related factors of nurse/auxiliary nurse staffing levels. None of these variables were statistically associated with the incidence of CDAD. These findings confirm results published by Vernaz et al. (33) which demonstrated no association between ABHRs and the incidence of CDAD. This finding is plausible due to the fact that ABHRs (and chlorhexidine) are not reliably effective against C. *difficile* spores (34). The inclusion of ABHRs and chlorhexidine in the analysis was, however, necessary, as these products can select for *C. difficile* spores due to their lack of sporicidal activity and thus may in fact facilitate the spread of *C. difficile* as hypothesized by some experts (5, 11). Within the present study, there was, however, no evidence of such facilitation.

The study design has several strengths, including the use of a robust analysis method which allows a comprehensive evaluation of the temporal relationship between explanatory variables and the incidence of CDAD. In addition, the study involved all patients hospitalized during the study period, with the exception of the pediatric patients, who were excluded since the WHO DDD system is not applied for this group of patients. Furthermore, data were collected as part of routine hospital practice and independently from the study; thus, selection and information biases are unlikely. At the Antrim

TABLE 4. Projections for the Antrim Area Hospital on the required usage of DDDs and on the number of patients needed to be treated to cause or prevent the occurrence of one CDAD case

Variable	No. of DDDs[a]	No. of patients[b]	Direction of effect[c]	Lag time[d] (mo)
Antimicrobials				
Expanded-spectrum cephalosporins	97	14	Positive	2
Broad-spectrum cephalosporins	55	8	Positive	2
Fluoroquinolones	261	37	Positive	3
Amoxicillin-clavulanic acid	659	94	Positive	1
Macrolides	545	78	Positive	5
Gastric acid-suppressive agents: H2RAs	966	NA[e]	Positive	1

[a] Number of DDDs needed in a given month to contribute to the occurrence of one CDAD case.
[b] For antimicrobials, this represents the number of patients needed to be treated in a given month to cause the occurrence of one CDAD case. This number was based on the assumption of an average treatment course of 7 DDDs.
[c] A positive direction of effect means that an increase in the mentioned DDDs and number of patients contributes to an increase of one CDAD case.
[d] Delay necessary to observe the effect.
[e] NA, not applicable.

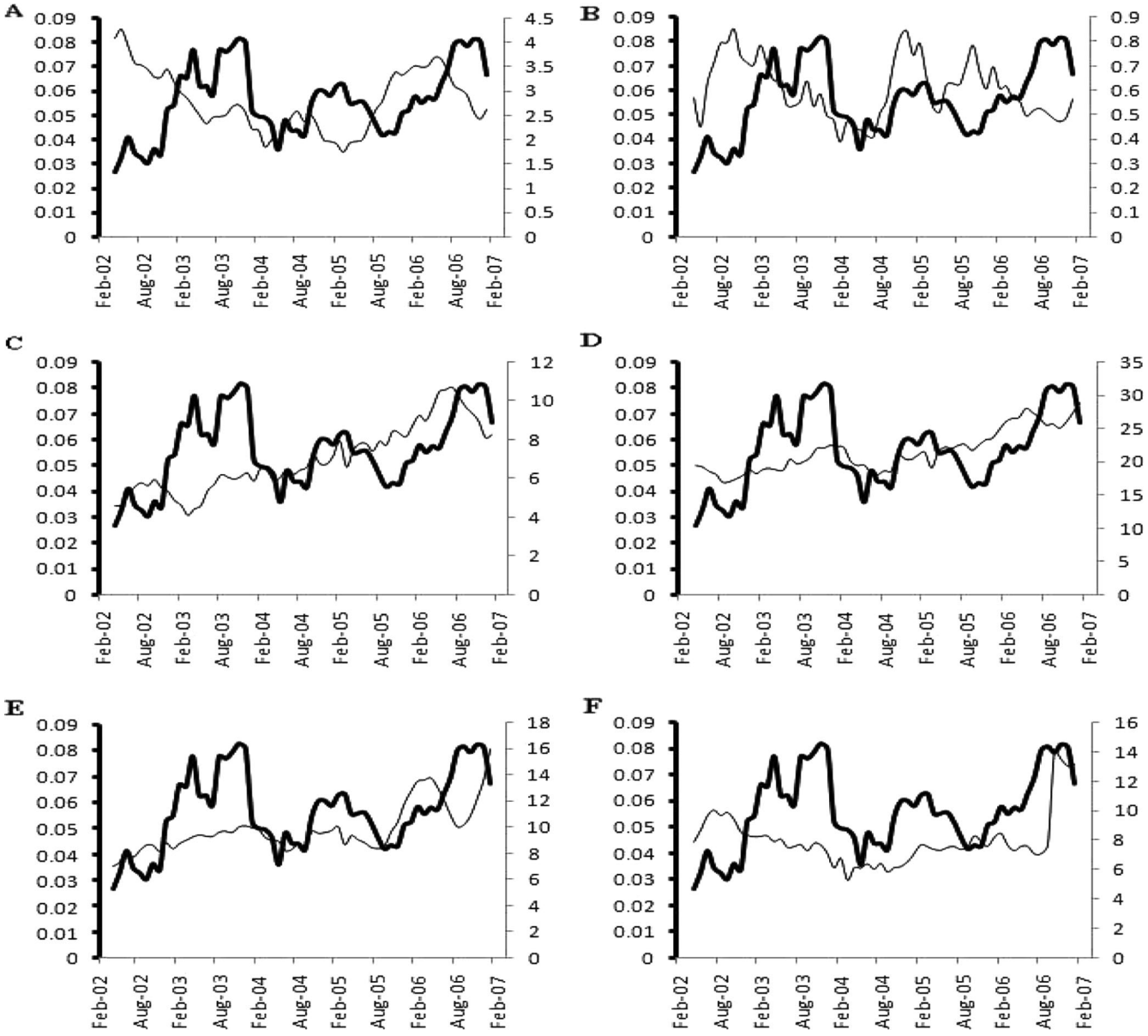

FIG. 1. Monthly CDAD incidence versus use of selected antibiotic classes and H2RAs, Antrim Area Hospital, February 2002 to March 2007 (thick line, CDAD, number of cases/100 bed-days, 5-month moving average, left *y* axis; thin line, antimicrobial use and H2RAs, DDDs/100 bed-days, 5-month moving average, right *y* axis). (A) Expanded-spectrum cephalosporins; (B) broad-spectrum cephalosporins; (C) fluoroquinolones; (D) amoxicillin-clavulanic acid; (E) macrolides; (F) H2RAs.

Area Hospital, infection control practices during the study period were in line with national and international guidelines. General hospital cleaning involved detergent and water; however, for patients in isolation for CDAD, detergent followed by a chlorine releasing agent was used. The policies were implemented in all clinical areas, and there was an ongoing program of audit of clinical practices, decontamination of clinical equipment, and environmental hygiene.

The study has some limitations. First, since the study was ecological and observational in design, it was not possible to control for different patient group characteristics and changes in patient population and case mix which may have affected the incidence of CDAD. Such parameters may be involved in the 22% of the variance which was not explained by our model. Second, associations demonstrated by quasiexperimental studies at the population level may not correlate with associations at the level of individual patients (13). Third, antibiotic resistance patterns in relation to CDAD were not available for the analysis as testing for CDAD antibiotic susceptibility was not a routine practice in the hospital. However, expanded-spectrum/broad-spectrum cephalosporins and fluoroquinolones (particularly ciprofloxacin) have poor activity against *C. difficile*, and macrolides have demonstrated only moderate activity against this pathogen (25, 27). Fourth, specimen ribotyping to identify the presence of the virulent *C. difficile* 027 ribotype was not carried out during the study period. A sample ribotype survey

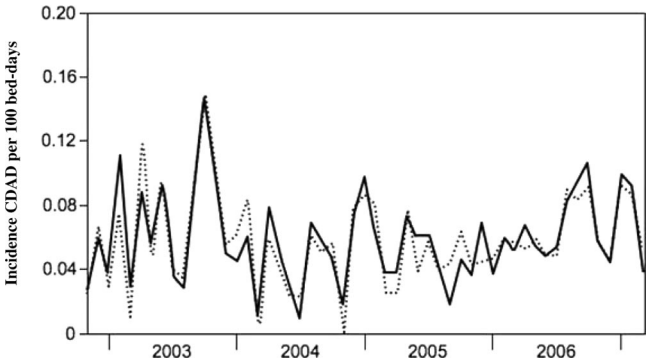

FIG. 2. Monthly CDAD incidence (solid line) and monthly sum of the lagged explanatory variables with their respective coefficients (dashed line) as identified in the multivariate time-series analysis model: expanded-spectrum cephalosporin use, broad-spectrum cephalosporin use (lag of 2 months), fluoroquinolone use (lag of 3 months), amoxicillin-clavulanic acid use, H2RA use (lag of 1 month), and macrolide use (lag of 5 months), Antrim Area Hospital, February 2002 to March 2007.

was, however, carried out across Northern Ireland from September to December 2006 (local data provided by Communicable Disease Surveillance Centre Northern Ireland); no cases of *C. difficile* 027 were identified. The first case of this ribotype in Ireland was reported in 2007 (17). Fifth, although it was not possible to control for the change in the hospital CDAD screening policy which took place in January 2005, clinical staff in the hospital have a high clinical awareness of the occurrence of CDAD, and as such the risk of missed cases is low. Finally, the number of CDAD cases was small. The research would have benefited if a larger sample size had been possible.

In conclusion, the present research attempted to clarify, in a comprehensive fashion, the combined role of antibiotics, gastric acid-suppressive agents, and infection control practices in the incidence of CDAD. The findings of this study highlighted a potential causal relationship between certain classes of antibiotics, H2RAs, and CDAD incidence. Whereas there is a significant body of research supporting a cause-effect relationship between antibiotic use and the incidence of CDAD, there is no consensus in relation to the association between gastric acid-suppressive agent use and CDAD. More research is needed to investigate the gastric acid-suppressive agents before limiting their use in hospitals. The findings confirm that the restriction of specific antibiotics could reduce the incidence of CDAD, but further prospective work is required to investigate the feasibility of restricting those antibiotics and to identify appropriate alternatives during restriction periods. The results of this research, however, can help hospitals to set priorities for restricting the use of specific antibiotic classes, based on the size-effect of each class and the delay necessary to observe an effect. Measuring the delay required to observe an effect following the restriction/use of particular antibiotics, which was possible using the time-series analysis technique, could be a possible way forward in determining the optimal time required for an antibiotic restriction policy.

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